



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Synaptic pathology at the neuromuscular junction in a mouse model of childhood motor neuron disease (spinal muscular atrophy)

Citation for published version:

Murray, LM, Thomson, D, Wishart, TM & Gillingwater, TH 2008, 'Synaptic pathology at the neuromuscular junction in a mouse model of childhood motor neuron disease (spinal muscular atrophy)', pp. 84-84.
https://doi.org/10.1111/j.1469-7580.2007.831_2.x

Digital Object Identifier (DOI):

[10.1111/j.1469-7580.2007.831_2.x](https://doi.org/10.1111/j.1469-7580.2007.831_2.x)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Publisher Rights Statement:

Available under Open Access.

Copyright © 1999–2013 John Wiley & Sons, Inc. All Rights Reserved.

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Mutations in the gene encoding a nuclear intermediate filament protein, lamin A, cause a spectra of human age-related diseases and premature ageing syndromes, affecting a number of somatic tissues including muscle, heart, adipose, bone, neurons and skin. One disease mechanism for lamin diseases proposes that lamin A mutations impair the control of adult stem cell proliferation via retinoblastoma protein (Rb) pathway, which has a critical role in the maintenance of mammalian stem cell populations. Recently, we demonstrated the role of lamin A in Rb-dependent cell cycle regulation and maintenance of proliferation in adult skin cells. To further understand the role of lamin A in longevity and maintenance of proliferation we studied its role in ageing of human dermal fibroblasts (HDF) *in vitro*, which undergo a progressive loss of proliferative cells and an accumulation of irreversibly arrested senescent cells during ageing.

Our results show that human fibroblasts aged *in vitro* acquire a range of aberrant nuclear phenotypes characteristic of progeroid human fibroblasts. Moreover, we show using three different biochemical techniques, including 2D-gel electrophoresis and mass spectrometry, glutathione blot assays and immunoprecipitation methods, that the C-terminal specific cysteine residues in lamin A undergo oxidative modifications (S-glutathiolation and other irreversible oxidative modifications) in senescent fibroblasts. These modifications inhibit the formation of higher-order disulphide-linked forms of lamin A in senescent fibroblasts as shown by cysteine cross-linking assays. In addition, during biochemical fractionation of senescent fibroblasts, these modifications led to a partial proteolysis of lamin A within its C-terminal domain. Consequently, lamin A fails to tether retinoblastoma protein (pRb) within the nuclei of senescent fibroblasts. Consistent with these findings, addition of extracts from senescent fibroblasts to a *Xenopus in vitro* nuclear assembly system caused oxidative modifications to C-terminal cysteine residues in *Xenopus* lamin LIII and inhibited nuclear lamina assembly and DNA replication.

Our findings suggest that lamin A acts as an oxidative stress sensor and is a central component of senescence signalling. We propose a novel model for ageing of human fibroblasts *in vitro* whereby the accumulation of oxidative damage to lamin A contributes to senescence signalling by de-stabilising the nuclear architecture. This novel model of ageing by lamin A redox state may explain the impaired maintenance of cells and tissues and decreased longevity in patients with lamin A mutations and may help develop future drug treatments based on anti-oxidant therapy.

26

Use of a student response system as a tool for lecturing in anatomy

S. Brettell,¹ C. Summerside¹ and S. McHanwell²

¹Quality in Learning and Teaching; ²Oral Biology, School of Dental Sciences, Newcastle University, UK

Student response systems are increasing in popularity as a tool to encourage participation in lectures. However, there is relatively little substantive literature on their effectiveness. Implementation and widespread use would involve a significant investment, both financial and in academic staff time, therefore Newcastle University ran pilot studies across the institution. Here we report the results of a pilot in teaching anatomy to speech sciences students. The research questions posed by this study were: does utilising the system in the lecture theatre improve the student learning experience;

how much time and effort does it take to integrate questions into existing lecture materials and has utilising the system in the lecture theatre had a positive impact on the teaching experience. Data was gathered on the student experience using an online questionnaire and lecture observations. With the system in use virtually all students responded to questions. This contrasts sharply with student reports of their behaviour in the absence of the system where only 46% of students said that they would answer if they were certain they were correct and 14% if they thought they may be correct. Students also reported discussing the options they chose with their peers. This may be a factor in their self-reporting of the use of the system increasing their learning. Qualitative comments were also positive, for example 'Once we saw the answers, it clarified concepts and the lecturer was able to see common mistakes and explain these points. Sometimes you don't realise you don't understand something until you make a mistake on a question.' and 'I think because you actually have to think about it during the lecture to answer the questions because without it you can just get away with passively making notes during the lecture and not actually take anything in'. We conclude that appropriate use of this system can have a positive impact on the lecture experience, both for staff and students.

POSTERS

P1

NG2-glia in the adult mouse brain: relations to neurons and astroglial

Rebekah Wigley,¹ Akiko Nishiyama,² Frank Kirchhoff^{3,4} and Arthur M. Butt¹

¹Institute for Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK; ²Department of Physiology and Neurobiology, University of Connecticut, USA; ³Neurogenetics, Max Planck Institute of Experimental Medicine, Germany; ⁴DFG Research Centre for Molecular Physiology of the Brain, Goettingen, Germany

A novel glial cell has been identified in the central nervous system (CNS) by the presence of the chondroitin sulphate proteoglycan NG2 on its cell surface. The precise role of these NG2-glia is not known, but in the developing CNS they are oligodendrocyte progenitor cells (OPCs) and differentiate into oligodendrocytes, the myelinating cells of the CNS. Furthermore, current evidence indicates that NG2-glia may also generate neurons and astrocytes under appropriate conditions. NG2-glia persist in large numbers in the mature CNS, which raises the possibility they may be a source of multipotent neural stem cells (NSCs). We hypothesise that glutamate released by neurons may regulate the differentiation capacity of NG2-glia, since they have been shown to contact synapses and respond to synaptically released glutamate. Here, we describe the relations of NG2-glia with astrocytes and neural elements in the mouse brain, making use of two transgenic lines in which NG2 drives the expression of DsRed, and glial fibrillary acidic protein (GFAP) drives the expression of enhanced green fluorescent protein (EGFP). Mice aged postnatal day (P)15 – 30 were killed by lethal injection of sodium pentobarbitone, in accordance with regulations issued by the Home Office of the United Kingdom under the Animals (Scientific Procedures) Act, 1986. Tissue was fixed in 4% paraformaldehyde and vibratome sections were cut of

the in the mid-sagittal plane at a thickness of 100 μ m. Sections were immunolabelled using antibodies to NG2, GFAP, calbindin (Purkinje neurons) and synaptophysin (synapses). Images were captured on a Zeiss 510 meta confocal microscope and analysed following deconvolution using Volocity 4.0.1 software (Improvision, UK). NG2-glia and astrocytes are organized in radial domains, but whilst the domains of the same cell type overlap very little, those between astrocytes and NG2-glia overlap completely. In the adult cerebellum, NG2-glia and Bergmann glia cover overlapping domains that encompass multiple Purkinje cells. The results demonstrate that NG2-glia are integrated into neuron-glia networks, but differences between NG2-glia and astroglia indicate they may form distinct communications pathways with different functions.

R.W. is supported by a BBSRC PhD studentship.

P2

Vulnerability of motor nerve terminals to hypoxia

B. Baxter, T. H. Gillingwater and S. H. Parson

Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, EH8 9XD, UK

Damage to neurons caused by ischemia and/or ischaemia-reperfusion injuries are now thought to contribute to the neurological defects seen as a consequence of surgery, cardio-pulmonary failure and stroke. There is growing evidence to indicate that the nerve terminal/synaptic compartment of neurons is more vulnerable to pathological stimuli than the axon and cell body, and therefore more likely to demonstrate functional and morphological changes in response to perturbation. To investigate if nerve terminals are especially vulnerable to ischaemic-reperfusion injury we have developed an ex-vivo model utilizing the mouse neuromuscular junction. Preliminary work has investigated the hypoxic element of ischaemia as this is believed to be the most damaging. In this model, isolated, intact tibial nerve and associated lumbrical muscles from the hind leg are maintained in a large volume of physiological saline/ringer bubbled with 95%:5% N_2 : CO_2 for hypoxic and 95%:5% O_2 : CO_2 for control and reperfusion conditions. Results show that 2 h of hypoxia followed by 2 h reperfusion can selectively trigger rapid disruption of motor nerve terminal morphology. The observed heterogeneity of the morphological responses to hypoxia, indicates that several mechanisms leading to nerve terminal disruption may be active and that these mechanisms may, at least in part, be controlled locally. It is likely that these morphological changes will have an adverse affect on synaptic function and connectivity, which may contribute to some of the punitive symptoms following ischaemia/ischaemia-reperfusion injury to the peripheral nervous system.

P3

Towards a novel multisegment foot model

Paolo Caravaggi,¹ Russell Savage² and Robin Crompton³

PREMOG lab, School of Biomedical Sciences, Liverpool University, UK

The basis of walking, possibly the predominant human activity, ultimately relies on the foot; hence it is of great interest to investigate its biomechanics and gain an insight into the optimizing principles underlying its kinematics. A new 9-joint 10-segment rigid body

model of the lower limb and foot is proposed. Instead of modelling the intricate net of ligaments spanning the articulations, these latter have been modeled as simple hinge joints with given values of rotational stiffness, damping and range of motion to simulate their mechanical behavior under physiological loads. An optimization approach is being evaluated to work out the joint axis location and orientation; it is reasonable to assume that given the kinematics governing the motion of 2 adjoining segments the ideal joint position is the one that minimizes the joint reaction forces. Here we apply the optimization method to the kinematics of the first metatarsophalangeal joint.

So as to track the segment trajectories during 'in vivo' movements, a 15-marker lower limb protocol has been identified. Preliminary kinematic data have been collected to drive and help optimize the computer model.

As it is the main passive structure sustaining the longitudinal arch, the plantar aponeurosis has been added to the model. This is of particular interest since its relation with the force at the Achilles tendon and the role of the windlass mechanism in raising the foot arch during the complete gait cycle has not yet been investigated in the literature.

With the purpose of obtaining an accurate representation of the foot/ground interface, a Visual Basic routine has been written to import the actual morphology of the surface of the sole of the foot from a laser scanner into multibody dynamic software. The algorithm reduces the point cloud to a grid of points, the density of which matches the pressure plate resolution to allow for experimental data comparison. Each point of the grid is used as the attachment location for spherical contact bodies connected to the overlying bone segments through spring/damper elements, the mechanical properties of which are currently being evaluated, using indentation test data from the literature. When the model is validated it will be possible to correlate dynamic pressure and local soft tissue deformations with lower limb kinematics for different loading conditions.

P4

Evidence that oligodendrocyte differentiation in the mouse brain is dependent on the inward rectifying potassium channel Kir4.1

V. E. Bay and Arthur M. Butt

Institute for Biomedical and Biomolecular Sciences (IBBS), School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK

The differentiation of oligodendrocyte progenitor cells (OPCs) into myelinating oligodendrocytes (OLs) is dependent on numerous factors. We have previously shown that OL differentiation is accompanied by a negative shift in the resting membrane potential, which depends on the timely expression of inwardly rectifying potassium channels (Kir). Studies in Kir4.1 knock-out mice (Kir4.1^{-/-}) have shown that there is severe hypomyelination in the absence of Kir4.1. Here, we have examined whether the loss of Kir4.1 and the resultant hypomyelination are due to the loss of OLs or blocked differentiation. Kir4.1^{-/-} mice, heterozygous (Kir4.1^{+/-}) mice and wild-type (Kir4.1^{+/+}) litter mates aged between postnatal day (P) 5 and P10 were killed by overdose of sodium pentobarbitone or cervical dislocation, in accordance with the Animals (Scientific Procedures) Act 1986. Optic nerves were rapidly removed and explants placed in culture for 10 or 20 days *in vitro* (DIV). Proliferation and maturation of OPCs was optimized by addition of human recombinant platelet derived growth factor (PDGF-AA) at 10 ng/mL for the first 3–4 DIV

and 0.5 mM dbcAMP thereafter for up to at least 10 DIV. Cultures were fixed in 4% paraformaldehyde and immunolabelled with stage-specific antibodies, namely NG2 for early and late OPCs, O4 for late OPCs and early oligodendrocytes and myelin basic protein (MBP) for mature oligodendrocytes. Cultures were examined using a Zeiss 510 meta confocal microscope and assessed by their morphological complexity, antigenic phenotype and the relative numerical density of OPCs and mature oligodendrocytes. The results indicate that in the absence of Kir4.1 OLs are decreased in number and appear abnormal in culture. We are currently examining changes in OPCs and cell death, but the initial results indicate that Kir4.1 is essential for normal OL differentiation. In future studies, we will test this hypothesis directly using transfection techniques to 'knock-in' Kir4.1 in Kir4.1^{-/-} OPCs and OLs, and 'knock-down' Kir4.1 in Kir4.1^{+/+} OLs and OPCs.

Supported by the BBSRC. V. Bay is supported by an IBBS PhD Studentship.

P5

Impact of caffeine, a human teratogen/embryotoxin, on connexin expression in the chick cardiomyocytes micromass (MM) and D3 embryonic stem cell (ESC) systems

B. Ahir and M. K. Pratten

Centre for Integrated Systems Biology & Medicine, School of Biomedical Sciences, Faculty of Health & Medicine, Queen's Medical Centre, Medical School, University of Nottingham, UK

Cardiomyocyte cells are electrically coupled by gap junctions, clusters of low-resistance multisubunit transmembrane channels composed of connexins (Cx). There are three major connexins, Cx40, Cx43 and Cx45. The expression of two of these, Cx43 and Cx45, which are present in cardiomyocytes, is known to be developmentally regulated. In addition, Cx43 and Cx45 play an essential role in cardiomyocyte differentiation during the development of the fetus of many species including, chicken, mouse, human and rat. In early embryo development, Cx43 plays an important role in the development of the outflow tract and coronary arteries, whilst Cx45 is involved in the development of endocardial cushions, indicating that Cx45 is important for the first contractions of the early embryonic heart. The study reported here investigates the idea that alterations in gap junction proteins are one of the mechanisms by which teratogens may act. Specifically, that molecule known to be teratogenic in the human may, by this mechanism, cause alterations in cardiomyocyte differentiation resulting in abnormal heart development. Thus, the possible effects of caffeine on gap junction protein (Cx43 and Cx45) formation was studied in chick cardiomyocytes in micromass culture. Five day old White Leghorn chick embryo hearts were dissected to produce a cardiomyocyte suspension in Dulbecco's Modified Eagle's Medium (DMEM). Also, D3 murine stem cells were induced to form embryoid bodies (EB's) upon removal of LIF, using the hanging drop method, and subsequently grown in culture to spontaneously form contracting cardiomyocytes. Cell cultures were incubated at 37 °C in 5% CO₂ (V/V in air) in the presence and absence of caffeine (200–1000 µM) and observations made for cellular differentiation (cell beating) at 24 h, 48 h and 144 h. Cell activity was assessed using the resazurin assay and total protein through the kenacid blue assay. Effects on Cx43 and Cx45 expression were analysed using immunocytochemistry. The results indicated that as the caffeine concentrations were increased up to 1 mM there was an increase in the cell beating observed and that this

coincided with and increase in the expression of Cx43 and Cx45. These experiments provide evidence that chick cardiomyocyte and ESC culture may be useful *in vitro* methods for mechanistic studies of perturbation of embryonic development.

P6

Microtubule plus-end cortical interaction and epithelial differentiation

D. A. Goldspink, G. Bellett, C. James and M. M. Mogensen

School of Biological Sciences, UEA, Norwich, UK

Epithelial differentiation involves a dramatic rearrangement of the microtubule cytoskeleton, which is vital for the normal function of specialized cell types. The microtubules are arranged in a centrosomally focused radial array in undifferentiated epithelial cells. During polarization and differentiation the microtubule cytoskeleton reorganizes into an apico-basal array anchored at apical non-centrosomal sites. A release and capture model has been proposed to be responsible for the generation of this array. In this model the microtubules are nucleated at the centrosome, released, translocated and captured at apical sites. Evidence from our lab suggests that microtubule plus-end cortical interaction is a vital intermediate step in the formation of apico-basal arrays. This interaction is likely to involve microtubule +TIPs (plus end-tracking proteins) such as CLIP-170, cortical receptors and motor proteins.

Our findings have revealed a microtubule-independent apical cortical pool of CLIP-170 in polarized MDCKII cells at potential plus-end capture sites. Interestingly, dynein also exists in distinct cortical clusters. We hypothesize that CLIP-170, cortical receptors and dynein play key roles in facilitating the assembly of the apico-basal array. The aims are to determine whether CLIP-170 is required for microtubule plus-end cortical capture and whether dynein mediates microtubule repositioning following centrosomal release. Depletion of CLIP-170 using siRNA in confluent ARPE-19 cells affected microtubule organization when challenged with nocodazole. Regrowth experiments showed compromised microtubule plus-end capture leading to an unfocused disorganized microtubule network. IQGAP1, a junction located protein, is a likely cortical binding partner for CLIP-170. CLIP-170 at microtubule plus-ends was shown to co-localize at the cell cortex with IQGAP1. Preliminary IQGAP1 depletion studies in ARPE-19 cells led to the formation of disorganized unfocused microtubules. We are currently investigating the effect of CLIP-170 and IQGAP1 depletion in polarized epithelial systems.

P7

Role of VEGF in diabetic angiogenesis: evaluation of human placental villous explant cultures

F. Sciota and L. Leach

School of Biomedical Sciences, Faculty of Medicine, University of Nottingham, UK

Vascular endothelial growth factor (VEGF) is the most potent primary inducer of angiogenesis currently known and is thought to be behind increased placental angiogenesis in pregnancies complicated by Type 1 diabetes. What in the diabetic milieu causes

the upregulation of VEGF remains unsolved and requires investigation. Suitable human culture models which allow long duration manipulation whilst maintaining a 3-D architecture and multiple tissue interactions is therefore desirable. The aim of this study was to evaluate the usefulness of chorionic villous explant cultures. Three to four placental villous explants (10 mg each; from normal term pregnancies) were incubated in 5 mL of oxygenated euglycaemic Medium 199 (with added 20% fetal calf serum and antibiotics) and agitated for 24 h at 37 °C. At 1, 4 and 24 h, explants were fixed with 1% *P*-formaldehyde for 30 min and then frozen in liquid nitrogen. Frozen sections were subjected to immunocytochemistry and the percentage of vessels showing immunoreactivity to VEGF and their intensity of labelling was estimated, using selective random sampling. Immunolocalisation of the endothelial-specific vascular endothelial-cadherin (VE-cadherin) was used as a marker. The explants showed no difference in the percentage of blood vessels showing immunolocalisation of VEGF between 0, 1 and 4 h (58.6%, 66%, 58.7%; $P > 0.05$). A statistically significant downregulation of VEGF was seen at 24 h (49.6%, $P < 0.01$). There were differences in intensity of labelling (graded 1–4) between the groups. At 0, 1 and 24 h, the majority of vessels showed a labelling index of < 2 . At 4 h, a higher proportion of blood vessels showed a labelling intensity of 4 which was significantly different from the other groups ($P < 0.001$). VE-cadherin localisation did not alter in all groups studied. Our data suggests that the chorionic villous explant culture system can be used for studies into VEGF expression after 24 h in culture. The increase in VEGF labelling between 0–4 h may be due to the initial stress response which led to the observed post-translational modifications of VEGF. Future studies into the effects of hyperglycaemia and hypoxia after 24 h duration of insult, using normal and diabetic placenta, may provide evidence of VEGF involvement in diabetic angiogenesis.

Funded by the ASGBI.

P8

Targeting NG2-glia in organotypic brain slices using an immunotoxin against the NG2 chondroitin sulphate proteoglycan

G. Leoni,^{1,2} M. Ratray² and A. M. Butt¹

¹Institute for Biomedical and Biomolecular Sciences (IBBS), School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK; ²Wolfson Centre for Age-Related Diseases, King's College London, UK

Novel glia have been identified in the central nervous system (CNS) by their specific expression of the NG2 chondroitin sulphate proteoglycan (CSPG) on their cell surfaces. These NG2-glia have poorly defined functions, but they are an abundant population of cells which contact nodes of Ranvier in the white matter and synapses in the grey matter where they respond to neuronal activity. Our overall aim is to selectively ablate NG2-glia in order to determine whether their loss affects CNS cytoarchitecture and function. We have previously demonstrated *in vitro* that an immunotoxin (ITxn) system against NG2 is effective in inducing selective cell death of NG2-positive cells. Here, we tested the efficacy of ITxn in ablating NG2-glia *ex vivo* in organotypic cultures of cerebellum slices from transgenic mice where the expression of the DsRed fluorescent protein is driven by NG2, or where the enhanced green fluorescent protein (EGFP) is driven by the astroglial glial fibrillary acidic protein (GFAP) promoter. Mice aged between postnatal day (P)7 and P15

were humanely killed by overdose of sodium pentobarbitone or cervical dislocation, in accordance with the Home Office Animals Scientific Act 1986. Brains were transferred to an HSB solution kept at 4 °C supplemented with glucose and coronal slices were cut at 300 µm using a tissue chopper. The slices were then placed onto membrane inserts and cultured at 37 °C using a 25% horse serum-containing medium supplemented with glucose and antibiotics, replacing the medium twice a week. The slices were maintained for up to 14 days *in vitro* (DIV) and ITxn was added for an exposure time variable from 2 to 7 days. The morphology and number of NG2-glia and astroglia were analysed at different DIV, in controls and ITxn treated slices. The ITxn was not effective in ablating NG2-glia *ex vivo* in organotypic brain slices, at a concentration that effectively killed NG2-positive cells *in vitro*. We also show that under our organotypic culture conditions, both NG2-glia and astroglia proliferate and acquire a reactive morphology. Also Bergmann glia underwent dramatic changes in culture, and we are currently examining the effects on neuronal cytoarchitecture. In conclusion, the ITxn and organotypic slice methods used in the present study are not good models for studying normal neuron-glia interrelationships, but may be useful for studying dynamic reactive changes in glia.

Giampaolo Leoni is supported by a PhD studentship from the Anatomical Society of GB and Ireland.

P9

Could the fat pad of the rat Achilles tendon enthesis organ function as an immune organ?

H. M. Shaw, R. M. Santer, A. H. D. Watson and M. Benjamin

School of Biosciences, Cardiff University, Cardiff, UK

Adipose tissue is a conspicuous, though greatly neglected component of entheses (bony attachments of tendons or ligaments) and a number of functions for this fat have been proposed, including stress dissipation and sensory perception. As adipose tissue elsewhere in the body is known to function as an immune organ, we have sought to determine whether the retromalleolar fat pad component of the Achilles tendon enthesis organ contains monocytes and/or macrophages which may play a similar role at attachment sites. The Achilles tendon enthesis organ was removed from male Wistar rats at 1 day, 1 month, 4 months, and 24 months of age (3 of each age), fixed in 4% paraformaldehyde, stored overnight in 10% sucrose buffer and cryosectioned. Sections were immunolabelled with the PAN macrophage/monocyte marker – CD68 (ED1), and inflammation marker – myeloid related protein (MRP) 14. Antibody binding was detected with a biotinylated secondary antibody using a Vector avidin/biotin kit. Labelling was developed with NovaRED and the sections were counterstained with Mayer's haematoxylin. In all animals and at all ages, CD68 positive macrophages were present within the retromalleolar fat pad. In aged (24 month) rats, such macrophages were additionally present within the sesamoid and periosteal fibrocartilages and on their bursal surfaces. Blood vessel-associated MRP14 labelling was also seen in the fat pads of 24 month rats. This enthesis-associated adipose tissue may therefore play a role in protecting the attachment site from infection and/or removing cellular debris from the retrocalcaneal bursa. If similar findings can be confirmed in human entheses, this may have implications for understanding the seronegative spondyloarthropathies – debilitating, rheumatic conditions in which the enthesis is generally considered to be the primary 'target organ'.

P10

Wnt signalling regulates transcription factor networks in vertebrate heart development

J. Martin and S. Hoppler

Department of Developmental Biology, University of Aberdeen, Institute of Medical Science, UK

The heart is the first organ to become functional in the vertebrate embryo; however the mechanisms regulating its formation are not yet fully understood. One of the most important and elementary steps in this process is the decision of mesodermal cells to assume a cardiac fate and subsequently differentiate into heart muscle. This decision is provoked by the interaction of signalling cascades and extra-cellular cues including the Wnt family of secreted signalling proteins. In this study we investigated the hierarchy of effects that β -Catenin (an important regulator of Canonical Wnt Signalling) and GATA6 overexpression alone or combined have on the formation of *Xenopus* hearts. This was achieved using mRNA injection of inducible, constitutively active constructs along with the potent pharmacological Wnt signalling agonist BIO. Embryos were injected with either β -Catenin-GR, GATA6-GR or with both constructs together in the presence or absence of dexamethasone. In separate experiments GATA6-GR injected and un-injected control embryos were treated with BIO. The effect these manipulations had on heart development were assessed by analysing gene expression of cardiac differentiation markers *Mlc2* & *Tnnc* along with the early heart precursor marker *Nkx 2.5*.

The results showed that overexpression of β -Catenin using either the β -Catenin-GR or the pharmacological reagent BIO leads to a down regulation of these heart markers, whereas GATA6-GR overexpression in the presence of dexamethasone leads to an increase in such expression. Overexpression of both β -Catenin and GATA6 results in a return to normal levels of expression in these markers suggesting that GATA6 is acting downstream of β -Catenin and is a relevant target of Wnt/ β -Catenin in heart development. FUTURE WORK: Further experiments are now being carried out in order to assess whether this regulation is direct or indirect. These experiments will make use of the already optimized BIO and GATA6-GR combined rescue experiment with the addition of Cyclohexamide a known inhibitor of protein synthesis. If GATA6 is a direct target rescued expression of cardiac differentiation markers effect will be observed.

P11

Synaptic pathology at the neuromuscular junction in a mouse model of childhood motor neuron disease (spinal muscular atrophy)L. M. Murray,^{1,2} D. Thomson,^{1,2} T. M. Wishart^{1,2} and T. H. Gillingwater^{1,2}¹Centre for Integrative Physiology & ²Centre for Neuroscience Research, University of Edinburgh, Edinburgh EH8 9XD, UK

Mounting evidence suggests that synaptic connections are early pathological targets in many neurodegenerative diseases, including motor neuron disease (Wishart et al., *J Neuropathol Exp Neurol* 65, 2006). A better understanding of synaptic pathology is therefore likely to be critical in order to develop effective therapeutic strategies. We have investigated synaptic pathology in the *Smn1*^{-/-};SMN2 mouse model of type I Spinal Muscular Atrophy (SMA: a childhood

form of motor neuron disease). We quantified synaptic innervation at the neuromuscular junction in a range of late-symptomatic (P5-P6) *Smn1*^{-/-};SMN2 muscle groups. Neonatal mice were killed by intra-peritoneal injection of sodium pentobarbitol and the transversus abdominis (TA) and levator auris longus (LAL) muscles dissected. Post-synaptic acetylcholine receptors were labelled by exposure to rhodamine-conjugated α -bungarotoxin, and neurons were labelled immunocytochemically with anti-150 kDa neurofilament antibodies. Synaptic pathology was quantified using a combination of fluorescence and confocal microscopy. There was a significant reduction in the number of fully innervated motor endplates in the LAL (~20% reduction) and the TA (~40% reduction) in *Smn1*^{-/-};SMN2 mice. We observed the presence of abnormal axon branching that was most prevalent in areas where synapse loss was less advanced. Significant intra-muscle variation existed for both synaptic loss and abnormal axon branching, in agreement with previous studies from adult-onset motor neuron disease (ALS). We also show that synapse loss in the wasted mouse model of early-onset motor neuron degeneration (which has a phenotype remarkably similar to *Smn1*^{-/-};SMN2 mice) proceeds in advance of axonal pathology, suggesting the presence of a distal axonopathy. Visualisation of synaptic ultrastructure in wasted mice indicated that synapses were lost via a mechanism distinct from Wallerian degeneration. We conclude that synaptic loss is a major pathological event in conditions characterized by early-onset motor neuron degeneration, including SMA.

P12

The potential of folic acid to prevent retinoic acid induced cardiac defects: an evaluation in chick cardiac micromass culture

S. Memon and M. K. Pratten

Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham, UK

Congenital malformations of the heart are major birth defects, affecting approximately 1% of the human population. Although genetic and environmental factors play an important role, either separately or in combination (multifactorial), still the cause in most cases remains unknown. Retinoic acid is used for the treatment of various dermatologic disorders. These retinoids when administered in excess during pregnancy can produce teratogenic effects on early heart development. Periconceptional supplementation with folic acid has been shown to help prevent neural tube defects. Various studies also support an association between multivitamins containing folic acid and reduction in the occurrence of congenital heart disease. The aim of this study was to detect the possible teratogenic effects of retinoic acid, in chick cardiac cells in culture and the possible preventive effects of folate on retinoic acid induced teratogenicity. Embryonic hearts were dissected from 5 day old white leghorn chick embryos to produce a cardiac cell suspension in DMEM culture medium. Cells were exposed to various concentrations of all trans retinoic acid (0.2–100 μ M) and folic acid (1–1000 μ M). End points for cellular differentiation were observational scores at 24, 48 and 144 h following explantation. Cell viability was established with resazurin and kenacid blue assays. Statistical analysis of the results was via one way ANOVA and Kruskal Wallis tests and *P*-value < 0.05 was considered significant. Retinoic acid significantly reduced cellular differentiation at and above 0.8 μ M (*P* value < 0.05), while cell viability was affected at higher

concentrations of $> 8 \mu\text{M}$ (P value < 0.05). Folate exposure led to no significant effects on any parameter (P value > 0.05). However folate (1 mM), when administered with varying concentrations of RA, could significantly reduce the effects of lower concentrations of RA, although it was less effective for higher concentrations. Supplementation with folic acid during pregnancy may be useful to prevent the teratogenic effects on heart development that may be brought about by retinoic acid.

P13

Development of cranial foramina in the chick embryo

S. Akbareian, A. Pitsillides and I. McGonnell

Veterinary Basic Sciences Department, Royal Veterinary College, London, UK

Cranial foramina are tiny holes which form in the skull during development. They are essential structures which allow the entry and exit of blood vessels and nerves through the skull.

Failure to form in the embryo and also closure in adulthood can lead to blindness, deafness and facial paralysis. Closure of these cavities also causes raised intracranial pressure which can be fatal.

The cellular and molecular mechanisms controlling foramina development have never been investigated. We have examined the formation of foramina in the chick embryo, using whole-mount alcian blue and alizarin red staining from D7 to D20. We have observed that foramina are initially very large but decrease in size as development continues.

Using H & E and Nissl staining on histological sections we have observed that foramina contain both nerves and blood vessels, as well as mesenchymal cells which become more sparsely distributed as development continues. We have further analysed these blood vessels and nerves using immunohistochemistry. Nerve bundles are labeled in all of the foramina examined. Interestingly we have discovered that blood vessels inside some of the foramina do not label with smooth muscle actin antibody and we suggest that this is due to them being part of a glomus body within the foramina.

We have also analysed patterns of cell proliferation and cell death in foramina at various stages from D4 to D12, using immunohistochemistry with PCNA antibody and TUNEL labeling. PCNA staining at D7 shows proliferative cells closely associated with the blood vessels, but proliferation is reduced as development continues. TUNEL labeling at D8 and D9 indicates apoptotic cells are present in blood vessel walls inside foramina. There is sparse distribution of TUNEL labeling in the mesenchyme inside foramina suggesting that apoptosis does not play a major role in 'mesenchymal clearing' of cells within the foramina.

These results are a primary indication of cell behaviour during development of these cavities and give new insight into how they may fail to form in the embryo.

Sponsored PhD Studentship from The Anatomical Society of Great Britain and Ireland.

P14

Repairing cartilage: can cranial neural crest cells help?

S. Ainsworth and D. J. R. Evans

Brighton and Sussex Medical School, Brighton, UK

A significant amount of vertebrate movement is reliant on synovial joints. The bones at a synovial joint are lined by a layer of articular cartilage which provides a smooth surface that reduces friction between bones during movement. Unfortunately during trauma and disease the cartilage may become worn away or damaged resulting in movement difficulties and pain. Treatments are wide ranging and include surgical intervention where new cartilage is transplanted into an affected joint. Unfortunately this approach has not been particularly successful as scar tissue often results and tissue lacks true integration. The vertebrate embryo has a number of cell populations that give rise to cartilage and it seems logical that such a cell population or one modified to act like it may be able to recapitulate chondrogenesis with more success. Cranial neural crest cells (CNCC) are known to produce facial cartilage in the embryo and so we are investigating whether such cells can also integrate into a limb developing joint when introduced into this new environment. First we have used a lacZ-encoded replication-deficient retroviral vector to label CNCC of stage 10 chick embryos. The results demonstrate that the midbrain CNCC give rise to a substantial amount of facial cartilage. Using this data we have cultured explants of midbrain neural tubes in different environments to assess the potential of these cells to form chondrocytes *in vitro*. Initial results suggest that medias supplemented with specific factors promote accelerated growth of the explants and produce the most varied cell types. Using the lacZ marker approach we have also injected regions of stage 28 developing chick limbs in order to identify a suitable region in which to subsequently introduce donor cell populations. Histology of labelled regions demonstrate that injecting into the foot plate region of limb results in cells labelled within the vicinity of the developing intertarsal joint. The next stage of this project is focussed at refining the location of the desired graft site and purifying suitable populations of donor cells.

P15

Functional and connexin expression studies on human omental arteries and veins isolated from pregnant women

S. Hammond, A. M. Mathewson, T. M. Mayhew and W. R. Dunn

School of Biomedical Science, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK

Maintaining optimum vessel tone is important in controlling blood pressure. In small arteries and veins various substances that cause vasodilation are released: these include prostacyclin, nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). Although the latter factor has yet to be identified, roles for gap junctions, potassium ions, hydrogen peroxide and epoxyeicosatrienoic acids have been proposed. Release of vasodilators is particularly important during pregnancy where blood pressure may decrease but cardiac output increases. Functional studies in human myometrial arteries from pregnant women have shown that EDHF release is mediated by gap junctions (Kenny et al., *Br. J. Pharmacol.* 136, 2002). Here, we have undertaken functional and immunochemical studies on human omental arteries and veins. Vasodilator responses to increasing concentrations of bradykinin were recorded in a pressure myograph before and after treatment with the nitric oxide synthase inhibitor-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M) or the gap junction inhibitor-carbenoxolone (10^{-4} M). Data are expressed as mean \pm SEM from n experiments. In

arteries in the presence of L-NAME, the maximal response (R_{max}) was $67.4 \pm 4.9\%$. In the presence of carbenoxolone + L-NAME, R_{max} was $40.8 \pm 11.1\%$, $P < 0.05$ (repeated measures two-way ANOVA with a Bonferroni post test) ($n = 5$). Immunofluorescence studies examining gap junction expression in human omental arteries from pregnant women showed strong reactivity for connexin 37 in the tunica media and for connexins 40 and 43 in the endothelium, reinforcing the proposal that gap junctions are involved in the EDHF response. Few data are available about the identity of EDHF in small veins and their role in controlling cardiac output and blood pressure. Initial functional studies in human omental veins from pregnant women have suggested that gap junctions do not mediate the EDHF response. In the presence of L-NAME, $R_{max} = 44.7 \pm 7.4\%$. In the presence of carbenoxolone + L-NAME, the response was not significantly different, $R_{max} = 35.7 \pm 5.5\%$ ($n = 3$). Future studies will examine connexin expression in omental veins and compare this with arterial connexin expression.

S.H. is supported by a Studentship from the Anatomical Society of Great Britain & Ireland.

P16

Clues about organogenesis and maintenance of the adult mouse adrenal cortex from analysis of mosaic patterns of expression of a 21-OHase/LacZ transgene

Su-Ping Chang,¹ John D. West¹ and Steven D. Morley²

¹*Division of Reproductive and Developmental Sciences, Genes and Development Group, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, UK;* ²*Centre for Reproductive Biology, Clinical Biochemistry Section, Queen's Medical Research Institute, University of Edinburgh Medical School, 47 Little France Crescent, Edinburgh EH16 4TJ, UK*

Labelling studies show that cells of the adrenal cortex migrate centripetally (towards the medulla) during maintenance of the adult organ. This might reflect inward movement of cells produced by stem cells near the periphery but stem cells have not yet been identified definitively in the adrenal cortex. Previously, the mouse steroid 21-hydroxylase-A gene promoter (21-OHase) has been shown to direct adrenal cortex-specific expression of the β -galactosidase reporter in 21-OHase/LacZ transgenic mice. Multiple independent 21-OHase/LacZ transgenic lines show a similar mosaic reporter expression pattern, probably reflecting stochastic transgene silencing. Analysis of these mosaic patterns of transgene expression provides insights into organogenesis and may help identify when maintenance of the adrenal cortex (by putative stem cells) begins. In the adult, the mosaic expression occurs as radial stripes, spanning the adrenal cortex and remains unchanged between 8 and 52 weeks. A similar radial striped pattern occurs in adult mouse chimeras and X-inactivation mosaics. The radial stripes may represent clonal lineages arising from stem cells during maintenance of the adult cortex. Stripes are not present in fetal 21-OHase/LacZ transgenic adrenal cortices but gradually emerge from a more randomly orientated mosaic pattern. If the adult stripes represent clonal derivatives of stem cells, the time when stripes emerge may provide clues about when stem cells begin to maintain the adrenal cortex. To identify when radial

stripes emerge, the mosaic LacZ expression pattern was characterised in adrenal cortices of 21-OHase/LacZ transgenics from fetal (E14.5) to weaning age (P21). Initially the β -galactosidase staining pattern appeared as clusters of spots. At fetal stages these clusters appeared more or less randomly orientated but discontinuous radial arrays of cells appeared gradually around birth (P0–P5). This change was not accompanied by notable net adrenal growth and further investigations are required to determine whether cell proliferation (balanced by cell death) or changes in cell shape or cell movement occur at this time. Continuous radial stripes emerged later (P7–P10) and by P14 stripes formed the dominant pattern. By P21 the pattern was identical to the adult, suggesting that stem cell maintenance of the adrenal cortex may have begun.

P17

Synthetic retinoid analogues induce model cell culture systems to differentiate down a neural lineage

V. B. Christie,^{1,3} J. Collings,² A. Whiting,² T. B. Marder² and S. A. Przyborski^{1,3}

¹*Biological and Biomedical Sciences, Durham University, South Road, Durham DH1 3LE, UK;* ²*Chemistry, Durham University, South Road, Durham DH1 3LE, UK;* ³*ReInnervate Ltd, Durham DH1 3HP, UK*

Vitamin A and its derivatives, collectively termed retinoids, are essential for many biologically important processes and are potent modulators of cell proliferation and differentiation in vertebrates. Retinoids elicit biological activity via retinoic acid receptors (RARs) which are localised in the nuclear envelope. The importance of retinoid function is now being realised in both embryonic neural development and adult neural homeostasis, alongside their great potential as chemo-preventative/chemo-therapeutic agents. The aim of this research is to study the role of both natural retinoids and synthetic retinoid analogues during induced differentiation of adult, embryonic and tumorigenic neural progenitor model systems. It is known that all-trans-retinoic acid (ATRA), a naturally occurring retinoid which activates all RAR subtypes, induces neural differentiation in several cell types. ATRA and its natural isomer 13-cis retinoic acid (13-cisRA) are currently used in certain tumour therapy programmes. The *in vitro* study of these natural retinoids, however, is complicated by their photo-isomerisation when used under standard laboratory conditions. For this study, we have synthesised ATRA retinoid analogues, EC23 and EC19. Unlike ATRA they do not isomerise readily in response to light or heat, therefore potentially making them advantageous in the *in-vitro* investigation into retinoid modes of action. Preliminary data showed that EC23 elicited similar cellular responses to ATRA when tested *in vitro*, inducing neural differentiation in all systems studied. Its potency was high, with an optimal working concentration being 10 fold lower than ATRA. EC19 appeared to induce a higher percentage of glia in the adult neural progenitor model and was also optimal at 10 fold lower concentrations than ATRA. EC19 appeared to inhibit the growth of neural cells in the neuroblastoma SH-SY5Y cell line. Further investigation is required to deduce the exact methods of action of these exciting retinoid analogues. This work will have the potential to aid research into pharmacological manipulation of neural differentiation and its associated receptors.

P18

Efficiency of lentiviral vector transduction in injured rat spinal cord: a stereological analysis

F. Toal,¹ E. Donnelly,² T. O'Brien,² J. P. Kelly,³ J. P. Fraher,⁴ P. Strappe,⁵ P. Dockery¹ and S. S. McMahon¹

¹Department of Anatomy, NUI Galway, Ireland; ²Regenerative Medicine Institute, NUI Galway, Ireland; ³Department of Pharmacology and Therapeutics, NUI Galway, Ireland; ⁴Department of Anatomy, University College Cork, Ireland; ⁵Brain and Mind Research Institute, University of Sydney, Australia

Traumatic spinal cord injury is a major cause of disability, mostly in young people. Stimulating the regeneration of axons is a key component of spinal cord repair that increases the chances for recovery of function. Spinal cord injury not only causes direct damage to the tissue but also results in a secondary injury where a glial scar forms around the site of injury forming a lesion. In order to achieve axonal regeneration following injury damaged neurons require neurotrophic factors to promote neurite extension. In injured CNS tissue neurotrophins can act to increase the intrinsic neuronal growth state by down regulating the growth cone response to inhibitory signals and increasing the degradation of extracellular inhibitory factors. One way to deliver neurotrophic factors to injured spinal cord tissue is the use of lentiviral vectors (Taylor et al., *J. Neurosci.* **26**, 2006). These vectors can stably integrate into the genome and provide long term gene expression. In this study we carried out preliminary experiments to investigate the efficiency of transduction of lentiviral vectors in injured spinal cord by injecting lentiviral vector expressing green fluorescent protein (Lenti-GFP) into six injured rat spinal cords following moderate contusion injury at T9. Lenti-GFP was injected immediately following injury and 1 week post injury. Spinal cords from these rats were fixed, frozen and cryosections were examined for expression of GFP. The volume fraction of GFP-expressing cells in injured spinal cords was estimated at both time points. Immunohistochemical staining was carried out to identify the GFP-expressing cells. In this study we found many of lenti-GFP transduced cells were astrocytic. Volume fraction of lesion at various time points following injury was also examined.

P19

Neuritogenesis in adult hippocampal neurons grown in growth-permissive versus inhibitory environments *in vitro*

C. B. Mellough,¹ A. Wood² and S. A. Przyborski¹

¹School of Biological and Biomedical Sciences, University of Durham, Durham, UK; ²Wyeth Neuroscience, 865 Ridge Rd, Monmouth Junction, New Jersey, USA

The inability of the adult central nervous system to regenerate following injury largely depends on the expression of myelin-associated inhibitors. These ligands are present before the glial scar has formed and bind to the p75^{NTR}-NgR receptor complex on regenerating neurons, causing growth cone collapse and axonal retraction. Progress is being made towards elucidation of the downstream events which result in growth cone collapse and

retraction of the axonal cytoskeleton. The majority of studies that investigate myelin inhibition employ neuronal populations derived from the postnatal developmental period, or which lie in close apposition to the regeneration-permissive peripheral nervous system *in vivo*. In this study, the effects of myelin-associated glycoprotein (MAG) on neurite outgrowth was assessed in a population of differentiating neurons derived from adult hippocampal neural progenitor cells. We show that MAG does not alter neural progenitor cell fate but, unlike their developmental counterparts, neurite outgrowth from differentiating neurons was significantly attenuated by MAG. We demonstrate that this effect can be partially overcome (by up to 69%) by activation of the neurotrophin, cAMP and PKA pathways or by Rho-kinase suppression. We also demonstrate that combining regeneration promoting methods elicits enhanced neurite outgrowth from differentiating neurons under myelin inhibitory conditions when compared with solitary application. This work pertains especially to the facilitation of neural repair in the compromised adult brain by endogenous mechanisms, such as the mobilisation and appropriate integration of host stem cells for functional replacement within depleted neuronal circuitry.

P20

***In vitro* haematopoietic potential of WT1 null murine ES cells**

T. J. Cunningham, G. Schofield and C. G. Miles

Institute of Human Genetics, Newcastle University, International Centre for Life, Newcastle upon Tyne, UK

The Wilms' tumour suppressor 1 gene (WT1) is a multi-isoform and highly conserved transcription factor that is essential for normal mammalian urogenital development and has been implicated in the development of numerous malignancies, including leukaemia. WT1 null mice die mid-gestation, specifically due to heart defects, but also fail to develop kidneys or a spleen. Due to the early lethality of the WT1 null mouse, and hence an impaired ability to study the effects of a WT1 null genotype on haematopoiesis *in vivo*, this study utilizes an ES cells approach. Two WT1 null ES cell clones were compared to wild-type ES cells during embryoid body (EB) differentiation, and their ability to generate haematopoietic lineages was assessed. WT1 null ES cells were morphologically identical to wild type, expressed classical pluripotency markers and differentiated normally into EBs with respect to size, growth rate, morphology and expression of genes marking the three primary germ layers as well as extraembryonic endoderm. Flow cytometry analysis revealed significantly lower expression levels of haematopoietic cell surface markers, most notably CD41 (day 6 EBs, $P < 0.005$, $n = 3$, Student's *t*-test). Functional *in vitro* haematopoietic colony-forming assays (StemCell technologies two-step *in vitro* differentiation procedure) also revealed a significant disruption to normal haematopoiesis in WT1 null EBs. To mimic the overexpression of WT1 seen in leukaemic cells, a novel WT1 minigene was stably introduced into ES cells using a lentivirus vector delivery system. Intriguingly, overexpression of WT1 also results in a reduction in haematopoiesis as determined by flow cytometry. In summary, WT1 null as well as WT1 overexpressing EB cells are impaired in their ability to differentiate into haematopoietic lineages, a contradictory finding that may be consistent with the fact that both WT1 mutation and overexpression can be associated with leukaemia.

P21

Isolation and manipulation of turkey (*Meleagris gallopavo*) primordial germ cells to test the feasibility of producing a genomic archiveA. J. Wade,¹ N. French² and G. W. Ireland¹¹*Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK;* ²*British United Turkeys, Hockenhull Hall, Tarvin, Chester CH3 8LE, UK*

Safeguarding valuable turkey strains used to produce commercial poultry is of great economic importance. Primordial germ cells (PGCs), stem cell progenitors of the gametes, exhibit a complex migratory pathway in the avian embryo moving from the germinal crescent, through the blood, hindgut mesentery and eventually settling in the gonads. Germ line chimeras have been produced for some avian species by injecting PGCs into a host embryo. Mating together germ line chimeras could reconstitute the original strains. This research aims to: (i) establish an efficient method for the isolation of turkey PGCs, (ii) identify the cells using known markers, (iii) demonstrate that PGCs can be cryopreserved and remain viable, (iv) show that isolated reinjected PGCs could repopulate the gonads of host embryos. Blood was withdrawn from stage 12 to 18 embryos using a handheld micropipette system. PGCs were isolated from embryonic blood using Ficoll density gradient centrifugation and identified using histochemical, Periodic Acid Schiff, and immunological, Ovomucin like Protein OLP-1 (Halfter et al., *Development* **122**, 1996) markers. PGCs were isolated from all stages but most efficiently from stage 14 and 15 embryos (26 ± 6 PGCs/embryo, mean \pm SEM). However, at stage 15, total PGC numbers were estimated to be at least tenfold greater from blood volume calculations. Whole mount 2-photon microscopy of OLP-1 stained stage 30 gonads showed 200–250 PGCs. Archived isolated frozen and thawed PGCs were 90% viable. Re-injected Vybrant CM-Dil-labelled PGCs were detected in whole mount stage 30 gonads by 2-photon microscopy at low frequency. Methods are currently being developed to test the *in vivo* efficiency of PGC ablation techniques so that the efficiency of germ line chimera formation can be increased.

P22

Stem cell-mediated gene therapy for muscular dystrophies: a study in NOD/SCID mice

Anabel S. de la Garza-Rodea, Ietje van der Velde, Manuel A. F. V. Gonçalves, Antoine A. F. de Vries and Shoshan Kna'an-Shanzer

Virus and Stem Cell Biology Laboratory, Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands

Duchenne muscular dystrophy (DMD) is a lethal muscle-wasting disease caused by mutations in the X chromosome-linked dystrophin gene. We investigated whether genetically corrected human mesenchymal stem cells (hMSCs) can be used as an alternative autologous cell source for DMD muscle repair.

MSCs are attractive candidates for such therapy as they are easily obtainable, can be expanded *ex vivo*, have a large differentiation potential and are readily transduced by viral vectors (e.g.

lenti- and adenoviral vectors). Furthermore, MSCs have the capacity to fuse with myoblasts *in vitro* and to contribute to muscle regeneration in dystrophic as well as experimentally injured muscles. We have introduced in our laboratory a model for muscle regeneration consisting of NOD/SCID mice which have one *tibialis anterior* (TA) muscle damaged by snake venom (cardiotoxin; CTX). Gene transfer into regenerating muscles has been assessed by transplanting, 24 h after CTX injection, hMSCs that were transduced *ex vivo* with a lentiviral vector encoding β -galactosidase (β -gal). Intramuscular injection of both CTX and cells has been performed under general anesthesia with Isoflurane. The animal experimental protocol has been approved by the ethics committee of the LUMC. Serial sections of the whole TA muscles harvested at 3, 7 and 18 days after treatment revealed the presence of single human cells expressing β -gal. At 30 days after treatment, in addition to the single cells, β -gal-positive myofibers were detected along the entire length of the muscle. These β -gal-positive myofibers differed in both diameter and length. Some were thin and shorter than 0.7 mm, others were of a larger diameter and reached up to 2 mm. Human dystrophin synthesis in the β -gal-positive myofibers is currently under investigation.

Our study confirms that in an *in vivo* muscle damage model, hMSCs can merge with myoblasts during the regeneration process and transfer genetic information into the newly formed myofibers.

P23

Investigation into the differentiation of motor neurons derived from human pluripotent stem cells and assessment of their function *in vitro*

Chendong Pan and Stefan Przyborski

School of Biological and Biomedical Science, Durham University, South Road, Durham DH1 3LE, UK

It is understood that retinoic acid (RA), sonic hedgehog (Shh) and bone morphogenic proteins (BMPs) play an important role in cell fate determination and the specification of inter-neurons and motor neurons along the dorsal-ventral axis in the neural tube. In this study, we investigated the function of these signalling molecules to instruct the differentiation of human pluripotent stem cells to form specific neuronal subtypes. TERA2.cl.SP12 embryonal carcinoma (EC) cells are a robust caricature of human embryogenesis and an accepted model of neural differentiation. Gene and protein expression analyses using RT-PCR, western blotting and immunocytochemical techniques indicated that human EC cells respond to RA, BMPs and Shh in a conserved manner and regulate neural transcription factors and structural proteins in a predicted way as cells commit toward the motor neuron phenotype. To assess the function of these differentiated neurons, we tested their ability to innervate skeletal muscle myotubes and induce muscle cell contraction. Myotubes contracted only when cocultured with neurons. The number of contractile events increased significantly when cells differentiated into motor neurons were cocultured with myotubes compared to cocultures with cells that formed interneurons. Staining for β -bungarotoxin showed positive staining in a pattern characteristic of buttons found in neuromuscular junctions. We also showed that muscle contraction could be manipulated pharmacologically: curare and atropine blocked myotube contraction, whereas acetylcholine and carbachol increased the number of contractile events. These results indicate it is feasible to control and direct the

differentiation of human stem cells and produce specific neuron subtypes *in vitro*. Furthermore, this system acts as a useful model to investigate the molecular mechanisms and signalling pathways that control neural differentiation in man.

P24

Presentation of extracellular matrix motifs by biomimetic substrates to control cellular attachment and differentiation

M. J. Cooke,¹ S. Phillips,² D. S. Shah,² D. Athey,² J. H. Lakey² and S. A. Przyborski¹

¹*School of Biological and Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, UK;* ²*Orla Protein Technologies Ltd, Nanotechnology Centre, Herschel Building, Newcastle upon Tyne NE1 7RU, UK*

Many different factors influence cellular behaviour. *In situ* cells are subject to several different signalling mechanisms, including; cell-cell interactions, soluble molecules and the local environmental geometry. *In vitro* model systems have been developed to recreate the *in vivo* environment and hence control and regulate cellular behaviour using known signalling molecules. *In vivo* the extracellular matrix (ECM) is composed of many such signalling molecules and these have been extensively studied. For example culture substrates can be coated with ECM components such as laminin, collagen and fibronectin to enhance cell adhesion and differentiation. Coating substrates with these molecules has proved invaluable in the growth and maintenance of cell types that do not readily attach to uncoated substrates. Furthermore substrates coated with these molecules have been shown to enhance neuritogenesis and increase process outgrowth. However the process of coating substrates in this manner is limiting in that the procedure is time consuming, has poor control and reproducibility. In this study, we in collaboration with Orla Protein Technologies, are developing novel biomimetic substrates to control cell behaviour *in vitro*. The effects elicited by molecules can be attributed to motifs within the molecules. We present different motifs derived from the ECM molecules laminin, collagen and fibronectin using a biomimetic substrate. In this way we mimic the interaction between cells and their local environment experienced in tissues. We have evaluated the application of this technology on cell adhesion and differentiation using the well established rat pheocytoma (PC12) cell line. Our data indicate that this technology can be used to control cell attachment to the substrates. We also demonstrate that the biomimetic substrates can be used to control process outgrowth of PC12 cells following nerve growth factor induced differentiation. We propose this system as an alternative method for consistent attachment and differentiation of cells.

P25

Connexin43 expression in cartilage progenitor cells and its possible role in cell differentiation

P. Marcus,¹ D. Bazou,¹ M. Howard² and C. W. Archer¹

¹*Cardiff School of Biosciences, Museum Avenue, Cardiff CF10 3US, UK;* ²*Smith and Nephew Research Centre, York Science Park, York YO10 5DF, UK*

Recent research has identified the presence of a progenitor cell population in the surface zone of articular cartilage that possesses an extended cell cycle capable of forming large colonies from a single cell and can engraft functionally into a variety of connective tissues. There has been a relationship noted in some cell types between the expression levels of connexins and the initiation of differentiation in progenitor cells. In this study, the ability of the progenitor cells to express connexin43 and form functional gap junctions was examined. Initially cells were suspended in an ultrasound trap to create the formation of cell aggregates which were immunolabelled for connexin43. To determine if these connexin molecules were capable of forming functional gap junctions, cells were labelled with the gap junction permeable cell tracker CMFDA. Three groups of cells were used: surface zone cells (containing approximately 0.5% progenitors and 99.5% differentiated chondrocytes), a clonal progenitor population and surface/progenitor mix. In each case a 1:3 ratio of labelled:unlabelled cells was used. Although the progenitor cells do express connexin on their surface, especially at regions of cell-cell contact, they do not appear to form functional gap junctions. Non-progenitor cells isolated from the surface zone of articular cartilage are able to form functional gap junctions almost immediately after aggregate formation. When these cells are mixed with unlabelled progenitor cells, dye transfer also occurs from the non-progenitor surface zone cells toward the progenitor cells suggesting that even though the progenitor cells cannot form gap junctions together, they are capable of communicating with differentiated cells types. It is possible that signals transferred by more mature cells in the surface could be a signal for the progenitor cells to undergo differentiation.

P26

Proteomic identification of neural stem cell biomarkers

Daniel J. Maltman^{1,2} and Stefan A. Przyborski^{1,2}

¹*School of Biological and Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, UK;* ²*ReInnervate Ltd, Durham DH1 3HP, UK*

Stem cell research suffers from a lack of protein markers which allow precise definition of cellular identity. Of the few antigens widely used to define neural stem/progenitor cells none are exclusive in these populations. Consequently accurate description of cellular status relies upon the measurement of multiple markers in combination. Comparative protein profiling offers rapid detection of multiple biomarkers making possible the high-throughput determination of cellular identity. Protein profiling via time-of-flight (TOF) mass spectrometry provides sample-specific peptide/protein mass signatures which can discriminate cell types without recourse to individual protein identification. Previously, surface-enhanced laser desorption ionization (SELDI)-TOF biomarker profiling has been successfully used in our laboratory to distinguish between control and differentiated cultures of a model stem cell system. Currently we are investigating the use of high accuracy matrix-assisted laser desorption ionization (MALDI)-TOF profiling to detect neural stem cell specific markers and to investigate proteins of neurogenic potential in conditioned media. Preliminary data indicate the potential to allow rapid evaluation of sample status, and to provide a lead into statistically significant protein candidates requiring further characterization. Findings may form the basis for the development of diagnostic immunological tools and novel growth supplements.

P27**Effect of retroviral mediated hNT-3 on hNSC differentiation**H. X. Lu,^{1,2} T. L. Parker² and Y. Liu¹¹*Medical School of Xi'an Jiaotong University, Xi'an, China, 710061;*²*School of Biomedical Science, University of Nottingham Medical School, Queens Medical Centre, Nottingham NG7 2UH, UK*

Genetic modification of neural stem cell (NSC) with neurotrophins is considered to be an effective approach to overcome the difficulty of poor survival and neuronal differentiation after transplantation. In our study, we genetically modified human fetal neural stem cells with retrovirus expressing human neurotrophin-3(hNT-3) to study the effect on NSC differentiation. Human fetal NSCs were incubated with recombinant retrovirus for 8 h and then cultured in DMEM/F12 serum free medium. Expression of hNT-3 was tested by PCR detection and dot blotting assay. The differentiation of transduced hNSCs was identified by immunostaining with specific antibodies Nestin, GFAP, Map2 and GalC at 7ds, 14ds and 56ds postinfection. Our results show hNT-3 gene was expressed successfully by transduced hNSCs and hNT-3 protein was secreted into medium for more than 14 days. Transduced hNSCs differentiated into 3 major neural cell types after seeding in DMEM/F12 with 1% FCS. In Retro-NT-3 transduced group, 70% cells were nestin positive at 7ds postinfection and approximately 50% of the cells retained nestin positivity after 56 days which is higher than Retro-C transduced hNSCs (24%). A two fold increase in Map2 positivity in NT-3 transduced hNSCs at 14ds and 56ds postinfection was observed with respect to Retro-C transduced cells. In conclusion, genetic modification with hNT-3 can promote the proliferation and neuronal differentiation of hNSCs.

P28**Characteristics of adult stem cells isolated from hair follicle and bone marrow**R. Emmerson,^{1,2} C. Jahoda¹ and S. A. Przyborski^{1,2}¹*School of Biological and Biomedical Sciences, University of Durham, South road, Durham, DH1 3LE* ²*Rinnervate Ltd, Durham, DH1 3HP, UK*

Stem Cells from the hair follicle (HFSCs) are an easily accessible population of cells that have potential for autologous replacement of damaged adult tissue. Evidence suggests that HFSCs possess a broad developmental potential and show some homologies with mesenchymal stem cells (MSCs) derived from bone marrow. However, the exact origin of these homologous cells remains unclear. The hair follicle represents a potential niche for a mesenchymal-like population of stem cells and contains epidermal stem cells in the bulge region and dermal papilla that play key roles in hair follicle development. The dermal sheath is of epithelial origin whereas the dermal papilla resides in the dermal component of the skin. Here we have compared the characteristics of rat clonal stem cell lines derived from either the hair follicle sheath (clone DS7) or papilla (clone DP9) with rat bone marrow-derived primary MSCs. Flow cytometric analysis showed the mesenchymal marker smooth muscle actin was expressed in all cells tested. Haematopoietic markers CD45 and CD34 were negative whereas mesenchymal markers CD44 and CD90 were expressed in both dermal clones and

MSCs. The more specific MSC marker, CD105, was not expressed on the clones but expressed in MSCs. Dermal clone DP9 also expressed the neural progenitor marker nestin, whereas this marker was absent in the dermal papilla clone DS7 and MSCs. When cells were grown under conditions to form aggregates, all three cell types expressed nestin, however they did not express the neuronal marker TuJ1. Neither of the HFSC clones were able to differentiate into bone whereas line DS7 was adipogenic and this was dependent on passage number. Our results indicate that clonal stem cells derived from distinct regions of the hair follicle have some homologies to MSCs derived from the bone marrow. However, in terms of aggregate formation and developmental potential, stem cells isolated from the dermal sheath appear to have more homology to MSCs than stem cells derived from the dermal papilla.

P29**Mesenchymal stem cell-derived soluble factors and their instructive effects on neural stem and progenitor cells**Steven A. Hardy,^{1,2} Adam P. Croft¹ and Stefan A. Przyborski^{1,2}¹*Department of Biological and Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, UK;* ²*ReInnervate Ltd, Durham DH1 3HP, UK*

Mesenchymal stem cell (MSC) transplantation in animal models of neurological disease has resulted in neurological improvement, however, the mechanism by which this occurs is under debate. Evidence suggests that MSCs have neurogenic potential and undergo differentiation into neural tissue to replace cells damaged in disease (trans-differentiation). These data are conflicting as others argue that MSCs do not trans-differentiate but instead fuse with host cells adopting their phenotype. However, both mechanisms are unlikely to fully account for the improvements seen. We are currently investigating a third mechanism whereby transplanted MSCs promote endogenous repair of neurologically damaged areas by the release of trophic factors and cytokines e.g. NGF and BDNF. We have shown that MSCs produce factors that instruct neural stem cells (NSCs) to adopt neuronal and glial lineages, and this is dependent on the developmental status of the MSC. Under standard culture conditions MSCs are negative for neural antigens, and produce factors which instruct NSCs to adopt a predominantly astrocytic fate. Conversely, we have shown that MSCs induced to form neurosphere-like structures, which are positive for neural antigens such as nestin and GFAP, instruct NSCs to adopt a predominantly neuronal fate. Such events are likely to contribute to the functional improvements observed in disease models following MSC transplantation. We are currently in the preliminary stages of identifying these unknown factors.

P30**Cerebellar ataxia: the establishment of an animal model of Purkinje cell damage in the rat cerebellum suitable for neural stem cell transplantation**H. X. Lu,^{1,2} H. Levis,¹ N. Melhem¹ and T. L. Parker^{1*}¹*School of Biomedical Science, University of Nottingham Medical School, Queens Medical Centre, Nottingham, UK;* ²*Medical School of Xi'an Jiaotong University, Xi'an, China*

The neurotoxin Penitrem A has been shown to specifically destroy Purkinje cells at defined concentrations. We have used this fact to create *in vitro* & *in vivo* animal models of cerebellar ataxias for neural stem cells (NSC) transplantation research. Adults (200 gr, $n = 6$) and neonates (6 days postnatal, $n = 48$) received IP injections of Penitrem A (2–4 mg/kg). After periods of 24 h to 72 h the rats were either killed by transcardiac-perfusion with 4% PFA under the Halothane anesthesia or by schedule one killing prior to organotypic slice preparation. Purkinje cells were identified either by H&E staining or by specific antibody to calbindin present on the Purkinje cell surface. Wax sections (5 μ m) were cut and the morphology and number of Purkinje cells were counted using a uniform random sampling method. Organotypic cerebellar slice culture was carried out on P6 rat pups using the Gahwiler method. After periods of 72 h–14 days culture the slices were fixed in 2% PFA and followed by calbindin immunocytochemistry. Data was analysed statistically and P -value < 0.05 was considered significant. The Penitrem A treatment resulted in 23.87% loss of Purkinje cells in neonates while there is no significant effect in granular cells as showed by the ratio of EGL/ML (1.14 vs 1.02 in pups and 0.73 vs 0.72 in adults). The significant decrease of Purkinje cells in neonates was observed in folia 7 (65.12%) and folia 9 (27.04%) ($P = 0.0186$ and 0.0198 respectively). On the contrary, there was no obvious change in folia 1–3 and folia 10. The slice results show 60.99% of Purkinje cells were lost in total. In conclusion, IP injection of Penitrem A can be used to produce Purkinje cell loss in both newborn and adult rat cerebellum which can be used to create cerebellar ataxic models suitable for neural stem cell transplantation both *in vitro* and *in vivo*.

P31

Comparison of the effects of periventricular injection of ibotenate in rats at postnatal days 5 and 7

A. Chen, N. Dimambro and G. J. Clowry

School of Clinical Medical Sciences and Institute of Neuroscience, Newcastle University, UK

Periventricular leucomalacia (PVL) involves hypoxia related damage to subcortical white matter in premature babies and is a common cause of cerebral palsy. Ibotenate (IBA) is an excitotoxic agent reported to cause PVL-like lesions when injected into the forebrain of neonatal rodents. We have compared injection of IBA at postnatal day 7 (P7) a stage of rodent corticospinal system development comparable to the stage of human corticospinal development vulnerable to PVL, and P5 when rodent oligodendrocyte precursor cells are most vulnerable to hypoxia and excitotoxicity. Surgical procedures were Home Office approved. Neonatal rat pups were anaesthetised and the skull was exposed and 2.5 μ g of IBA in 1 μ L of saline (saline only for controls) was injected 1 mm either side of the midline between the frontal and parietal skull bones at a depth of 2 mm below the cortical surface. Analgesia was maintained and the pups kept warm before being returned to their mother 3 h after surgery. Animals were eventually killed by transcardial perfusion with fixative under terminal anaesthesia. Immunohistological examination at P14 showed that IBA injection at P5 lead to areas of hypomyelination and cyst formation in the periventricular corpus callosum. There was concomitant localised loss of neurofilament immunoreactivity, astrocytosis and microglial activation, although the cortical grey matter remained intact.

However, treatment at P7 produced little or no hypomyelination, although the size of the lateral ventricles increased. From P28 onwards, rats were tested for corticospinal function by testing successful forelimb reaching and retrieval of food rewards. All rats improved with age, but by P42 there was a highly significant difference (two-tailed t -test $P < 0.01$) between IBA treated rats (P5 and P7) and controls, but there was no difference between saline treated controls and normal animals. Histological examination following testing revealed no difference in overall forebrain cross sectional area between experimental conditions, but that the lateral ventricles were significantly larger in IBA treated animals than controls, especially in P7 animals (two tailed t -test $P < 0.01$). P5 animals consistently displayed reduced myelin immunoreactivity in the corpus callosum compared to controls whilst myelination of the striatum and lower layers of the neocortex appeared normal. P7 animals showed unpredictably patchy and diffuse hypomyelination. We conclude that injection of IBA at P5 provides the most satisfactory model for PVL which we will use for exploring the possibilities of neonatal brain repair by transplantation of stem cells.

P32

Inducing the differentiation of embryonal carcinoma (EC) stem cells using natural and synthetic retinoids: screening, characterization and mode of action

C. E. Bridgens,^{1,2} J. H. Barnard,² J. C. Collings,² E. B. Cartmell,² H. M. Goss,² A. Whiting,² T. B. Marder² and S. A. Przyborski^{1,3}

¹School of Biological and Biomedical Sciences, Durham

University, UK; ²Department of Chemistry, Durham University, UK;

³ReInnervate Limited, Old Shire Hall, Old Elvet, Durham DH1 3HP, UK

Retinoids are a group of natural and synthetic molecules that are structurally and/or functionally analogous to all-*trans*-retinoic acid (ATRA), the active metabolite of vitamin A. ATRA regulates a broad range of essential processes during mammalian embryogenesis and adult homeostasis, including cellular differentiation, proliferation and apoptosis. Accordingly, it is hoped that retinoids may be used in a wide range of therapeutic applications, if it is possible to overcome the serious side effects observed when these compounds are administered at efficacious concentrations.

We have commenced a study directed at the synthesis of stable alternatives to ATRA and the investigation of the *in vitro* molecular pathways that regulate cell development in response to natural and synthetic retinoids. A small library of synthetic retinoids were designed and prepared and each test compound was then applied to TERA2.cl.SP12 human embryonal carcinoma (EC) stem cells at a concentration of 10 μ M for up to 28 days. Morphological observation and flow cytometric analysis were used initially to screen the compounds for the ability to induce cell differentiation. Characterisation of the differentiated cultures has been performed by western blot analysis and immunocytochemical localisation of antigens associated with neural and non-neural phenotypes.

In agreement with previous studies, synthetic retinoids that possess the same pharmacophore as ATRA induced the differentiation of EC stem cells. We observed the development of both neural and non-neural cell types in response to all compounds, though

the cultures show a variation in differentiation efficiency, termination of proliferation and the different proportions of terminal cell types. The most potent compounds can up-regulate antigens associated with neural phenotypes at comparable rates and in similar patterns to that which is observed in response to ATRA. Investigations into the mechanisms by which synthetic retinoids behave in the cell and induce differentiation, compared to the natural compounds, are underway. These compounds are more stable than ATRA, which is susceptible to photo- and thermal-isomerisation under laboratory conditions, and thus provide a more convenient and reliable reagent to modulate reproducible differentiation in cultured stem cells.

P33

Conditional depletion of parenchymal β 1-integrins in the adult murine liver

Fabio Quondamatteo,^{1,2} Ann-Kristin Brönnecke² and Tina Krone²

¹Department of Anatomy, NUI Galway, Ireland; ²Department of Histology, University of Göttingen, Germany

Beta-1 integrins mediate most cell-matrix interactions which may in turn be essential to the tissue. We characterized hepatic deletion of β 1-integrin in mx-cre conditional (floxed) mice after induction by means of 3 injections of poly IC in adult mice bearing floxed b1-integrin genes (kindly provided by the group of Prof. Reinhard Faessler, MPI Munich). Analysis was carried out 3 and 8 weeks after the first injection. Three weeks after induction it was possible to achieve efficient deletion of the β 1-integrin-gene, visualized by Southern Blot and localized in the tissue using immunohistochemical detection of β -galactosidase (activated as a reporter gene by the β 1-deletion). In the presence of deletion of the β 1 gene, β 1-integrin RNA (analyzed by in situ hybridization) was absent in the majority of hepatocytes and β 1-integrin, localized using two different antibodies, was no longer detectable in the parenchyma. β 1-Integrin depletion in parenchyma correlated with loss of regular lobular structure. Distribution of fibronectin and type IV collagen was not altered. Eight weeks after induction, less extensive deletion was evident. β 1-integrin-mRNA was detectable in a significant part of the cells. Use of polyclonal antibody revealed augmented parenchymal deposition of β 1-integrin in comparison to tissue 3 weeks after induction, but reduced presence of the protein in comparison to controls. The monoclonal antibody, in contrast, did not stain liver tissue 8 weeks after induction, neither in floxed nor in control mice. Eight weeks after induction, the liver tissue of floxed mice showed histological signs of proliferation and distribution of fibronectin and type IV collagen in the parenchyma was irregular. In conclusion, efficient deletion of β 1 integrins in adult liver tissue can be achieved 3 weeks after induction in this model. This indicates that the hepatic physiological turnover of β 1-integrins is less than 3 weeks. We also show that β 1-integrins are essential for the maintenance of the adult liver structure. Our results also suggest that 8 weeks after poly IC injection, replacement of liver cells may determine partial re-appearance of β 1 integrins at the gene, mRNA and protein level. Also, poly IC may possibly induce post-translational alterations in hepatic β 1-integrins 8 weeks after administration.

P34

Novel cell culture device for 3-dimensional cell growth and enhanced cell function

Maria Bokhari,^{1,2,3} Ross Carnachan,¹ Neil Cameron¹ and Stefan Przyborski^{2,3}

¹School of Biological and Biomedical Science and ²Department of Chemistry, Durham University, South Road, Durham DH1 3LE, UK;

³ReInnervate Limited, School of Biological and Biomedical Science, Durham University, South Road, Durham DH1 3LE, UK

The structure and function of cultured cells are dramatically affected by the micro-environment in which they are grown. Traditionally, two dimensional (2-D) polystyrene surfaces are used to support cell growth *in vitro*; however such surfaces do not enable the most favourable cell growth and function. A more thorough understanding of cell biology and cell-cell interactions requires three dimensional (3-D) culture systems that more closely represent the natural structure and function of tissues *in vivo*. Here we present a novel cell culture device that provides a 3-D environment for routine cell culture. We have developed a polystyrene scaffold which exhibits a well defined and uniform porous micro-architecture and have adapted these three-dimensional scaffolds for cell culture and/or tissue engineering applications. These scaffolds are readily adaptable to many different types of tissue culture plasticware including 6- and 24-well plates. These culture devices are pre-fabricated, sterile, and easy to use and are handled in a similar manner to standard 2-D plastic-ware. This material is manufactured through a high internal phase emulsion polymerization route and its surface chemistry can be varied to enhance cell adhesion and viability. We have exemplified the application of this technology by growing hepatocytes and osteoblasts. The performance of the above cells grown on 3D is enhanced compared to functional activity of cells grown on 2-D plastic. In a further application we have developed an organotypic model of mammalian skin consisting of a stratified sheet of epidermal keratinocytes grown at the media/air interface on a layer of dermal fibroblasts and collagen gel within the scaffold. This system enables long term growth and maintenance of polarised epithelia that closely resembles native skin. This technology can be used to investigate the function of skin epithelial cells in a broad range of applications, including basic science, development of pharmaceuticals and assessment of compound toxicity. The incorporation of thin membranes of porous polystyrene to create a novel device has been successfully demonstrated as a new 3-D cell growth technology for routine use in cell culture.

P35

Differentiation of the proliferative zones of the human neocortex during cortical plate formation

N. Bayatti, J. F. H. Ward, S. Lindsay¹ and G. J. Clowry

School of Clinical Medical Sciences, Institutes of Neuroscience and

¹Human Genetics, Newcastle University, UK

The proliferative zones of the developing mammalian neocortex comprise a ventricular (VZ) and subventricular zone (SVZ). In the rodent, the SVZ appears halfway through cortical plate (CP)

development and is relatively small. In primates, the SVZ appears at the start of CP formation, grows into a large and complex structure which is also the source of inhibitory interneurons and glia. We have made a detailed study of the human VZ and SVZ between 8 and 16 weeks post-conceptional age (PCW). Foetal brains obtained from the MRC-Wellcome Trust Human Developmental Biology Resource, collected following British national guidelines with maternal written consent and ethical approval from the local Health Authority, were dissected and fixed with buffered 4% paraformaldehyde solution. Paraffin embedded and frozen brain sections were immunoperoxidase stained or processed for *in situ* hybridisation for a number of molecular markers. At 8–9 PCW both the VZ and SVZ exhibited strong expression of Ki-67 and the transcription factor PAX6 throughout, but post-mitotic neuronal markers such as MAP2, TBR1 and NEURO D were mostly confined to the SVZ. By 10 PCW Ki-67 expression revealed two separate layers of proliferation, one present at the ventricle/VZ boundary, and the other at the VZ/SVZ boundary. GFAP, a radial glia marker, was present throughout the VZ and SVZ. From 12.5 GW, the VZ was present but relatively small, but the SVZ was expanded and split into outer and inner cell rich zones (ISVZ and OSVZ) separated by a cell poor zone containing tangentially orientated fibres. Within the proliferating layers, PAX6 was universally expressed, TBR2 at higher and more distinct levels in the ISVZ than OSVZ, while TBR1 and NEURO D exhibited widespread lower expression levels throughout the SVZ. In the rodent, cortical neurogenesis primarily involves asymmetric generation of cells from Pax6-positive radial glia with cell bodies in the VZ, with symmetric division of Tbr2-positive intermediate progenitor cells in the SVZ later in CP development. Tbr1 positive neurones are confined to non-proliferative zones. In the human, the situation appears to be different, with transition from PAX6 to TBR2 positive cells and from TBR2 to NEURO D or TBR1 expression occurring within a structurally complex SVZ from the earliest stages of development.

P36

Human therapeutic dose-levels of an aminoglycoside antibiotic gentamycin sulphate mildly but transiently affect the testicular functions and spermatogenesis in mice

K. Narayana

Department of Anatomy, Faculty of Medicine, Kuwait University, Safat, Kuwait

Gentamycin sulphate (GS) is used in the treatment of gram-negative bacillary infections, including those caused by *Pseudomonas aeruginosa*. The present study investigated the effects of GS on male reproductive functions in mice. Male Swiss albino mice (9–12 weeks old) were treated i. p. with 3 mg/kg and 6 mg/kg body weight for 10 days at intervals of 24 h ($N = 6/\text{dose/sample time}$). Mice were sacrificed by over dose of anaesthesia (Pentobarbitol sodium) on 21d, 35d, and 70d following the last exposure. One testis/mouse was processed for histopathological evaluation. Another testis was homogenised for estimation of testosterone and lactate dehydrogenase levels. Cauda epididymis was minced in 2 mL phosphate buffered saline (pH 7.35) pre-warmed to 35 °C and filtered through 80 μ nylon mesh. The smears were prepared from the filtrate and stained with PAS-hematoxylin or only with 1% eosin Y. Spermatozoa were observed for head and tail abnormalities and expressed as percentage incidences. The sperm count was

conducted in Neubaur's improved counting chamber and expressed as millions/mL. The percentage incidence of motile sperms was estimated according to the standard procedure. Data were expressed as mean \pm SD and statistically analysed by one way ANOVA followed by Bonferroni's post hoc test and P -value < 0.05 was considered significant. Gentamycin sulphate did not affect the body weight or the testis weight. Significant increase was seen in tubular diameter on 21d ($P < 0.01$), but only at 6 mg/kg on 35d ($P < 0.05$) although the epithelial thickness did not show any difference. Qualitatively, GS induced only minor structural changes in the testis. Sperm abnormalities increased on 21d at both dose-levels and on 35d, only at 6 mg/kg ($P < 0.05$). However, the sperm count was decreased only on 35d ($P < 0.001$) whereas the sperm motility was decreased on 21d and 35d in a dose-dependent pattern ($P < 0.01$ – 0.001), but no effect was seen on 70d. The testosterone level was decreased only on 35d in a dose-dependent pattern ($P < 0.01$), whereas the lactate dehydrogenase activities increased on 21d and 35d ($P < 0.01$). In conclusion, GS is cytotoxic to the testis and mildly affects the testicular structure and spermatogenesis in mice at human therapeutic dose-levels.

P37

Human umbilical vein endothelial cells (HUVEC) derived from type 1 diabetic pregnancies show enhanced hypertrophic response to VEGF

L. Leach and K. Loong

School of Biomedical Sciences, University of Nottingham, UK

Increased angiogenesis is a feature of feto-placental vessels in Type-1 diabetes with placental and fetal macrosomia a common occurrence. VEGF is a potent pro-angiogenic growth factor and has been shown to contribute to hypertrophy in different cell types. The aim of this study was to investigate the effect of VEGF on growth of HUVEC from Type 1 diabetic pregnancies. Concomitantly, the fate of β -catenin, an adhesion molecule important for cell cycle regulation, was monitored.

Freshly isolated HUVEC (2×10^5 cells/mL) from Type 1 diabetic pregnancies ($n = 3$) and normal pregnancies (control; $n = 3$) were grown to confluence in full medium (with added 20% FCS). Cells were serum starved (4 h, $t = 0$) and both groups were stimulated with VEGF (10 ng/mL) for 24 and 48 h. Intracellular distribution of β -catenin was monitored by immunofluorescence. Cell number, cell height and surface area (SA) were measured using phase and scanning confocal microscopy. Statistical significance was determined by two-way ANOVA and post-hoc tests.

For both groups, a reduction in cell numbers was observed with increased duration of VEGF ($P < 0.0001$). At $t = 0$, HUVEC from the Type 1 group had a larger cell SA (22%). After 48h VEGF, the SA of Type 1 group were significantly larger (139% increase from $t = 0$); normal HUVEC showed a 96% increase ($P < 0.01$). Cell height at nuclear regions (HN) showed no significant difference in cells from Type 1 diabetic group ($t = 24$; $t = 48$), however there was a decrease in HN in normal HUVEC ($P < 0.01$). A statistically significant loss of β -catenin from cell-cell contact regions to cytoplasmic (24 h) and nuclear regions (48 h) were seen in the Type 1, but not normal group suggesting a greater vulnerability of β -catenin to VEGF signalling here.

In conclusion, HUVEC from Type 1 diabetic pregnancies have intrinsic differences in growth pattern and an heightened hypertrophic

response to low levels of VEGF. β -catenin may be part of the mechanism behind this growth.

Funded by ASGBI.

P38

Age- and gender-associated changes in intima to media ratio (IMR) of mesenteric and coeliac arteries

B. Manoharan and R. C. Aland

School of Biomedical Sciences, University of Queensland, St Lucia, Australia

Intima-media ratio (IMR) indicates degree of intima thickening as a ratio of the media associated with infiltration of lymphocytes and lipid, cholesterol and calcium accumulation during formation of atherosclerotic plaques. IMR measurements in carotid arteries are currently utilised successfully as prognostic indicators and risk profiles to model the occurrence of occlusive cardiovascular disease and the course of atherosclerosis. Gut artery atherosclerosis has shown to be linked to the occurrence of mesenteric vascular diseases and mesenteric ischaemia, especially in the elderly (Hansen et al., *J. Vasc. Surg.* **40**, 2004), however no valid prognostic indicators, such as IMR, exist to model this. Thus, this pilot study investigated age and gender related differences in the coeliac and mesenteric arteries as described by IMR. Location specific changes of IMR and possible associations between IMR and prevalent and incident cardiovascular disease (CVD) were also investigated. The relevance of the IMR parameter in relation to atherosclerotic progressions linked to occlusive mesenteric vascular diseases was also explored. Dissection and measurements in 65 cadavers for the superior mesenteric artery, inferior mesenteric artery, splenic artery and common hepatic artery were performed before histological processing employing Millers & van Gieson's for elastic fibres and Gomori's trichrome. Computational image analysis was carried out and statistical analysis performed using Student's *t*-test, linear regression and Pearson's correlation. Males were shown to have larger mean IMR in the SMA and IMA than females ($P < 0.05$) and female SMA IMR was found to be associated with existence of CVD. No association between IMR and age was observed, however weak negative correlations were observed for the SMA IMR. It is suggested that females are more likely to have lower IMR values due to the anti-atherogenic qualities of 17- β oestradiol.

P39

A stereological study of the structural changes in hyaline cartilage in a model of rheumatoid arthritis

K. Thompson,¹ D. Brennan,¹ S. Glynn,¹ P. Dockery¹ and D. P. Finn²

¹*Department of Anatomy NUI, Galway, Ireland;* ²*Department of Pharmacology & Therapeutics NUI, Galway, Ireland*

The chronic inflammatory condition of rheumatoid arthritis (RA) is characterised by pathological cartilage destruction at the joint interface. In the present study, an arthritic condition analogous to that of human RA was induced in a group of Sprague Dawley rats via the adjuvant *Mycobacterium butyricum*. All procedures had appropriate ethical committee approval. Cartilage samples were

derived from hallux of the hind paw of two groups of male Sprague Dawley rats: A control group ($n = 4$), and an adjuvant-induced arthritis group ($n = 6$). The extent of the damage to the tissue, caused by arthritis, was investigated using light microscopic and stereological methods. The parameters that were assessed were: cartilage height h_{cart} , Vv matrix : cartilage, lacunar (chondron) volume weighted mean volume (Vv). The groups were redefined into a control group ($n = 4$), and the test group was further subdivided into those animals that did not/did display macroscopic evidence of joint swelling T1 ($n = 3$) and T2 ($n = 3$). One-way Analysis of Variance (ANOVA) was performed followed, where appropriate, by post hoc Tukey's tests. There was no significant difference in the height of the cartilage under study between the three experimental groups. There was a significant decrease in the Vv matrix: cartilage with treatment ($P < 0.027$). A subsequent Tukey's test revealed that both treated groups were smaller than the control group but not different from each other. The volume weighted lacunar/chondron volume increased in the arthritic groups ($P < 0.001$). A subsequent Tukey's test revealed that the control group had the smallest volume, followed by T1, with volumes largest in T2. This study has demonstrated quantifiable structural changes in articular cartilage in a model of rheumatoid arthritis. The data suggest that there is a relationship between the degree of structural change in articular cartilage and the macroscopic manifestation of inflammation.

Funded by: NUI Galway Millennium Fund.

P40

Using microarray analysis to identify candidate genes specifying digit identity in the chick wing

L. A. McDonald, M. F. Fisher and C. Tickle

Department of Cell and Developmental Biology, University of Dundee, Dundee, UK

Over the past 10 years, there has been an acceleration in the discovery of genes involved in vertebrate limb development, in particular those which control digit number and pattern. It is now well established that digit patterning across the anteroposterior axis of the vertebrate limb depends on Shh signalling. However, although Shh is crucial in determining the number and pattern of digits which form, relatively little is known about genes that are expressed in response to Shh and how expression of these genes is then translated into digit anatomy. We have capitalised on the increasing availability of genomic resources and used Affymetrix microarrays to screen for genes expressed in specific digit primordia in the chick wing. In collaboration with Ark Genomics, we have compared the transcriptional profiles of the primordia which will give rise to digit 2 and digit 4 in HH27 chick wings. 210 genes were found to be expressed at significantly higher levels in digit 2, and 150 in digit 4. An initial group of genes was chosen to verify differential expression via *in situ* hybridisation. It was found that Lhx9 and Neuroligin-2, for example, were highly expressed in digit 2 at HH27, while Glypican-1, Mesenchyme Forkhead-1 and Fibulin-1 were highly expressed in digit 4.

Optical Projection Tomography (OPT) was used to visualise the detailed expression patterns of these genes in 3-dimensions and compare them to expression patterns of genes already known to be involved in digit specification (e.g. Tbx3). This research should uncover new genes involved in digit anatomy and help to define the complete digit patterning process.

P41

Using rapid prototyping to complement traditional anatomical education

R. G. Thomas,¹ J. M. Delieu,¹ N. W. John¹ and M. Mahon²

¹University of Wales, Bangor, Wales; ²Keele University School of Medicine, Keele, UK

The traditional way of imparting anatomical knowledge has always been through the use of cadavers and prosections. However, ethical and financial issues, and a demise in the numbers of donated cadavers may lead to a detrimental effect on medical students gaining anatomical knowledge. We are developing an intuitive learning environment for anatomy education that complements traditional approaches and computer based learning materials. Taking advantage of the latest 3D computer graphics components and Rapid Prototyping (RP) technology, together with functionality such as virtual resections, we provide the learner with new tools to aid their understanding. RP is the practice of taking 3D virtual models and creating a physical equivalent. Typically rapid prototyping machines construct models layer by layer, which are then attached to each other by a process such as gluing, or fusion using a laser. The models tend to be made from a plastic material, although some machines may use paper, cardboard or a metal. Rapidly prototyped models can be produced to faithfully reproduce anatomy segmented from CT and other medical data. In our system, we segment the anatomy of interest from a patient's CT data scan e.g. a tumour of the liver. A RP model of this is then generated, which becomes the user interface for the anatomy student. Students hold and manipulate the RP model, which is tracked using magnetic tracking technology. As the model is manipulated, a volume rendering of the anatomy is displayed on the screen or through a head mounted display. The student can focus on the particular area of interest, and if required, can manipulate a virtual clipping plane so that they can see inside the organ. Further applications of RP modelling could also help students understand some of the difficult concepts in embryological anatomy.

P42

'Measure for Measure – Prosector's Labours Cost': the relative costs of running an Anatomy course based on different primary modes of teaching

M. K. Pratten and T. M. Mayhew

Anatomy Teaching Group, School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham, UK

Several factors have influenced the development of medical curricula worldwide during the last few decades and led to reductions in 'expensive' activities and the freeing of time for other relevant subjects. There has been a particular impact on the time and resource devoted to Anatomy teaching. Moreover, the advent of new Medical Schools and alternative or improved teaching tools (e.g. computer-based software, medical imaging) and the trend towards problem-based learning (PBL) have influenced Anatomy teaching in more traditional institutions and led to the concept of teaching without cadaveric material. However, whilst, it is true that cadaver-based teaching is costly, instituting alternative teaching

modes might also be costly. Surprisingly, there is little hard information about relative costs of different modes and no detailed comparison of the set-up or running costs of teaching by different methods. There are three primary modes by which Anatomy might be taught: student dissection, use of prosections or cadaverless. All might still make use of skeletal material, medical images, plastic models, surface anatomy and computer packages. Here, we provide a detailed cost-analysis for converting from a dissection/prosection-based course (the mode currently employed by the vast majority of Medical Schools worldwide) to either a prosection-only or a cadaverless course. Costs are based on 280 students taught in duplicated split classes of 140 students. Analysis is based on realistic costs for purchasing extra sets of skeletal material, models, images and, where appropriate, for generating extra prosections. Analyses include the maintenance and ancillary staffing budget to run each course based on replacement levels reported by other Medical Schools. Set-up costs were estimated at around £190k for a prosection-based course and £320k for a cadaverless course. Compared to the current dissection-based course, maintenance costs, increased 90% per annum for a prosection-based and 3% for a cadaverless course. Whilst we believe that decisions should be made on educational grounds alone, we conclude that there is no financial incentive to drive Anatomy teaching away from dissection.

P43

Demonstration of a student response system as a tool for lecturing in anatomy

S. Brettell,¹ C. Summerside¹ and S. McHanwell²

¹Quality in Learning and Teaching, and ²Oral Biology, School of Dental Sciences, Newcastle University, UK

Elsewhere in this meeting we report upon the results of a study examining the effectiveness of student response systems as lecturing tools in anatomy teaching. Here we demonstrate the system employed in that study from a staff and student perspective. A variety of commercial systems are available for collecting student responses and opinions during lectures and small group discussions. The system employed at Newcastle is TurningPoint, which integrates with Microsoft Office for display and reporting purposes. Possible pedagogical uses are to allow students to respond to direct questions, to stimulate discussion and to highlight common misconceptions to teaching staff. The demonstration will show a variety of question types and pedagogical uses.

P44

'To disassemble or dissemble: that is the question' – a review of Anatomy as it is currently taught in the UK and overseas

T. M. Mayhew and M. K. Pratten

Anatomy Teaching Group, School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham, UK

At the end of 2006, Faculty Management Committee at Nottingham Medical School decided to reconsider whether to continue to offer dissection to medical undergraduates. Consequently, we decided to conduct a survey of the way in which Anatomy is currently

taught in the UK and overseas and a cost-analysis of different courses based on [a] student dissection, [b] prosections alone and [c] cadaverless teaching. This report deals with these teaching modes rather than other aspects of delivery (regional vs. systematic, traditional vs. problem-based). Its aim was to clarify facts in light of the perception that dissection elsewhere is being abandoned. The survey made use of recent literature and the solicited views of colleagues and embraces current teaching in the UK, Australia, France, Germany, Ireland, North America and Switzerland. Main findings were: [1] Cadaver-based teaching continues in the overwhelming majority (> 88%) of Medical Schools (MSs). Of 5 new MSs in the UK, only one (the Peninsula) has elected to adopt cadaverless teaching. The consortium CME at Lancaster also teaches without cadavers. [2] Students learn in different ways and so a cadaver-based course presenting material in a variety of ways (including sectional and medical images, computer packages, living anatomy, models and bones) is likely to provide a richer and

more valuable experience for students as a whole. [3] Time devoted to practical (exploratory or skills-based) activities vs. didactic or knowledge-based teaching varies but most MSs retain some dissection or demonstrator-assisted prosection. [4] Reservations have been expressed about the costs of staffing and resourcing, and the educational value, of moving exclusively to use of prosections but we found no firm cost-analysis data favouring either dissection or prosection alone. [5] Concerns have been raised about increases in litigation claims and the possible consequences of loss of course attractiveness. [6] Innovations have been introduced in the absence of any evidence-based or cost-benefit analysis. [7] At a recent RCS conference, there was consensus that dissection should be used as a medium of instruction. In light of lack of hard information on cost comparisons, our companion report offers a cost-analysis for moves away from a dissection + prosection course to ones based on use of either prosections alone or cadaverless instruction.